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Fatty acid metabolism by the osteoblast☆

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ABSTRACT

The emergence of bone as an endocrine organ able to influence whole body metabolism, together with comorbid epidemics of obesity, diabetes, and osteoporosis, have prompted a renewed interest in the intermediary metabolism of the osteoblast. To date, most studies have focused on the utilization of glucose by this specialized cell, but the oxidation of fatty acids results in a larger energy yield. Osteoblasts express the requisite receptors and catabolic enzymes to take up and then metabolize fatty acids, which appears to be required during later stages of differentiation when the osteoblast is dedicated to matrix production and mineralization. In this article, we provide an overview of fatty acid β-oxidation and highlight studies demonstrating that the skeleton plays a significant role in the clearance of circulating lipoproteins and non-esterified fatty acids. Additionally, we review the requirement for long-chain fatty acid metabolism during post-natal bone development and the effects of anabolic stimuli on fatty acid utilization by osteoblasts. These recent findings may help to explain the skeletal manifestations of human diseases associated with impaired lipid metabolism while also providing additional insights into the metabolic requirements of skeletal homeostasis.

1. Introduction

The osteoblast, derived from a mesenchymal progenitor present in marrow and the periosteum, is the specialized cell tasked with the synthesis, secretion and assembly of the mineralized, collagen-rich matrix that composes bone tissue. Essential to this function is the development of an abundant and well-defined rough endoplasmic reticulum that can produce and package extracellular matrix proteins during osteoblast maturation [1,2] as well as the capacity to harvest significant amounts of chemical energy to fuel this intensive process [3]. Hierarchical analyses of ATP consumption indicate that protein synthesis, even at levels much lower than would be expected of an active osteoblast, is the most energetically demanding cellular process. As much as 30% of oxygen-coupled ATP production may be dedicated to protein production with the equivalent of 4 ATP molecules required to form each new peptide bond in a growing polypeptide [4,5]. Synthesis of the 3 polypeptides that compose a single type I collagen molecule would therefore be expected to require more than 17,000 ATP equivalents. Surprisingly, detailed investigation of the interplay between intermediary metabolism and bone cell activity has only recently regained the field's attention [6]; likely a result of worldwide epidemics of obesity, diabetes, and osteoporosis and the recognition of comorbidities among these conditions [7–9] as well as the emergence of bone as an endocrine organ that can influence whole body metabolism [10–12].

Studies conducted more than 50 years ago suggested that the osteoblast harvests energy via the metabolism of glucose. In vitro comparisons of glucose uptake by metaphyseal bone slices and liver explants revealed greater uptake in bone samples but a much lower level of oxygen consumption, which implied that glycolytic metabolism is dominant to oxidative metabolism of glucose in the osteoblast [13–16]. Neuman and others suggested that acid production via this metabolic paradigm could facilitate the solubility of calcium and phosphate ions in bone's extracellular fluid by lowering the pH [17–20]. More recent studies, utilizing more carefully characterized osteoblast cell models and cultures of primary cells, have confirmed the conversion of glucose to lactate by osteoblasts [21–25]. Additionally, molecular genetic studies demonstrated that glucose uptake via the Glut1 transporter is required for early osteoblast commitment and regulates the turnover of the master osteogenic transcription factor, Runx2 [26].

By comparison, the utilization of fatty acids by osteoblasts to fuel bone formation has received relatively little attention. The catabolism of fatty acids yields more energy than the metabolism of glucose as each cycle of β-oxidation (described in detail below) has the potential to generate 17 ATP. The complete oxidation of palmitate (C_{16}), the most common fatty acid in animals, can therefore be used to produce ~131 ATP, whereas the complete oxidation of a glucose molecule can yield just 38. Osteoblasts can oxidize fatty acids and early studies

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suggested that this might account for as much as 40% to 80% of the energy yield of glucose utilization [27]. More recently, we [28] demonstrated that fatty acid oxidation increases dramatically as osteoblasts mature in vitro such that the level of catabolic activity in mineralizing osteoblasts was 3-fold higher than proliferating cells. Moreover, pharmacological inhibition of β-oxidation in vitro impairs osteoblast differentiation [28], while carnitine supplementation to enhance lipid oxidation capacity is associated with an increase in collagen synthesis [29].

In this article, we provide a review of the experimental evidence that osteoblasts utilize fatty acids. We begin with a brief overview of the oxidative pathways by which cells catabolize fatty acids and then highlight potential mechanisms of fatty acid acquisition by osteoblasts. We describe the effect of genetic inhibition of long-chain fatty acid oxidation on bone mass and the regulation of lipid utilization by osteo-anabolic signals. Finally, we conclude with a brief summary of defects in bone metabolism associated with human conditions related to impaired lipid metabolism. For the sake of focus on potential anabolic effects of fatty acid metabolism in bone, we do not discuss the complex effects of high fat diets or oxidized lipids on the skeleton.

2. Overview of fatty acid metabolism

The cellular and physiological metabolism of lipids is critical for maintaining energy balance, membrane dynamics, and the generation of lipid signaling molecules among others. This is accomplished by both the generation of lipids de novo and the ingestion of dietary lipids, some of which mammals do not have the enzymatic machinery to synthesize. Genetic defects in many of the genes encoding this machinery are associated with human disease (described below) that represent stark reminders of the importance of lipid homeostasis.

Dietary lipids are packaged in the intestinal epithelial cells as chylomicrons. These large lipoprotein particles are trafficked first through the lymphatic system, which bypasses the liver, and then engage lipoprotein lipase on the endothelial surfaces of target tissues. Very low-density lipoproteins generated and secreted from the liver are metabolized in a similar manner. Lipoproteins can be taken up by receptor-mediated endocytosis at the target cell or alternatively fatty acids liberated by lipoprotein lipase at the endothelial cell surface can be taken up by the target tissue. While the detailed biophysical uptake and shuttling of free fatty acids remains controversial, fatty acids must be first activated by their ligation to Coenzyme A (CoA) by one of 26 Acyl-CoA Synthetases (ACS) encoded by the human genome to be utilized by further metabolic pathways [30,31]. Nonesterified fatty acids have low solubility within cells. The activation of fatty acids to acyl-CoAs traps fatty acids in cells, makes them more soluble in aqueous solution and generates the high energy thioester to enable downstream acyltransferase reactions. The ACS reaction generates PPi and AMP, therefore every fatty acid activated requires the replenishment of 2 ATP. Following their activation into acyl-CoAs they can then be shuttled into a multitude of anabolic or catabolic pathways. When the incorporation of fatty acids exceeds cellular requirements they can be shuttled and stored in lipid droplets in almost all cells as triglyceride or cholesterol ester for later use.

ATP generation is largely accomplished by the β-oxidation of fatty acids within mitochondria, with access to the mitochondrial matrix where the enzymatic machinery is located providing regulatory control over utilization. The transport of long chain fatty acids, the most abundant species that are made and ingested by humans, is mediated by the duel carnitine palmitoyltransferase (Cpt) enzymes located on the outer and inner mitochondrial membranes (Fig. 1A). Cpt1 located on the outer mitochondrial membrane generates acyl-carnitine from cytoplasmic acyl-CoAs. The acylcarnitines then traverse the inner membrane via the Carnitine-Acylcarnitine Translocase. Within the matrix, Cpt2 located on the inner membrane regenerates acyl-CoAs from the acylcarnitines. The regulation of import is mediated in large part from the allosteric inhibition of Cpt1 by malonyl-CoA, the committed step in fatty acid synthesis [32]. Cytoplasmic malonyl-CoA is generated by carboxylation of acetyl-CoA via highly regulated acetyl-CoA carboxylase (ACC) and utilized by Fatty Acid Synthase (FASN) to generate palmitate. The carbon for de novo fatty acid synthesis is largely from TCA cycle derived citrate. Fatty acid synthesis, therefore, is an efficient method to store carbohydrates. Excessive carbohydrate intake, particularly fructose, is highly lipogenic.

Acyl-CoAs are used to generate ATP and reducing equivalents (NADH and $FADH_2$) by the β-oxidation machinery in the mitochondrial matrix. In each cycle of the β-oxidation reactions, 2 carbons are removed from the carboxyl end of the acyl-CoA to form acetyl-CoA (Fig. 1B). The four step β-oxidation process involves: 1) the α,β dehydrogenation of the acyl-CoA to yield an enoyl-CoA with a trans double bond between the α and β carbons and $FADH_2$; 2) hydration of the α, β-unsaturated acyl-CoA to β-hydroxyacyl-CoA; 3) the oxidation of β-hydroxyacyl-CoA to form β-ketoacyl-CoA and NADH; and 4) the thiolysis of β-ketoacyl-CoA to yield acetyl-CoA and an acyl-CoA shortened by 2 carbons. One molecule of C_{16}-palmitate undergoes 7 cycles of the oxidative process to generate 8 acetyl-CoA molecules, 7 NADH, and 7 $FADH_2$. Acetyl-CoA enters the TCA cycle, while NADH and FADH are utilized in the electron transport chain to generate ATP. Mono- and polyunsaturated fatty acids must be isomerized and reduced before they can be fully processed by the β-oxidation machinery, but several cycles of β-oxidation may proceed before these reactions. For instance, the monounsaturated fatty acid oleate may undergo 3 cycles of β-oxidation to yield 3 acetyl-CoA before the isomerase enzyme converts the cis double bond to trans.

Short and medium chain fatty acids are also primarily oxidized in mitochondria, but very long chain fatty acids must first be chain shortened in the peroxisome. The peroxisomal β-oxidation machinery cannot fully oxidize fatty acids but will generate acetyl-CoA and octanoyl-CoA. These metabolites can be transferred to mitochondria for further oxidation by both carnitine-dependent and carnitine-independent mechanisms. Defects in peroxisomal fatty acid oxidation often cause devastating disease due in large part to an accumulation of inappropriately long or branched fatty acids. Peroxisomes are also important for the metabolism of branch-chained fatty acids as well as collaborating with the ER in the generation of dicarboxylic fatty acids via ω-oxidation.

3. Fatty acid uptake by osteoblasts

Skeletal lipid uptake has only recently been examined as the in vitro studies highlighted above that identified fatty acids as a potential energy source for osteoblasts did not examine uptake in the context of an intact organism. Using ^{125}I-labelled chylomicron remnants, Neimeier et al. [33] compared skeletal uptake of intravenously administered lipoproteins to other lipid-avid tissues and demonstrated a significant role for bone in lipoprotein clearance. Chylomicron remnant uptake by the femoral diaphyses was second only to liver and reached a level that was greater than that in the heart, kidney, and muscle. Additional studies using fluorescent-labelled remnants and electron-microscopy studies revealed that uptake was localized to osteoblasts. These data accord with in vitro findings that demonstrated a requirement for serum lipoproteins for normal osteoblast proliferation [34].

Kim et al. [35] performed similar analyses after the delivery of radiolabelled bromo-palmitate (a non-hydrolyzable isoform of the fatty acid) or oleate by gavage. Uptake of both tracers was evident in the femur, tibia, and calvaria at levels comparable to the quadriceps femoris muscle, but was only one-tenth the amount taken up by the liver. As these 3 bones represent only a fraction of the total biomass of the skeleton, it is reasonable to assume that bone also plays a role in free fatty acid clearance. Similar results were obtained by Bartelt et al. [36]. Kim et al. [35] predicted that fatty acids taken up by the skeleton are likely to be processed for the generation of ATP as in vivo measures of de novo lipid synthesis by bone were low (approximately a third of gonadal adipose tissue).

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